



Early Journal Content on JSTOR, Free to Anyone in the World

This article is one of nearly 500,000 scholarly works digitized and made freely available to everyone in the world by JSTOR.

Known as the Early Journal Content, this set of works include research articles, news, letters, and other writings published in more than 200 of the oldest leading academic journals. The works date from the mid-seventeenth to the early twentieth centuries.

We encourage people to read and share the Early Journal Content openly and to tell others that this resource exists. People may post this content online or redistribute in any way for non-commercial purposes.

Read more about Early Journal Content at <http://about.jstor.org/participate-jstor/individuals/early-journal-content>.

JSTOR is a digital library of academic journals, books, and primary source objects. JSTOR helps people discover, use, and build upon a wide range of content through a powerful research and teaching platform, and preserves this content for future generations. JSTOR is part of ITHAKA, a not-for-profit organization that also includes Ithaka S+R and Portico. For more information about JSTOR, please contact support@jstor.org.

THE RESPIRATORY MECHANISM IN CERTAIN AQUATIC LEPIDOPTERA¹

By
PAUL S. WELCH

INTRODUCTION

According to one of the current theories, insects arose from a terrestrial ancestry and the aquatic habit, wherever manifested, was secondarily acquired. The general application of this theory to all aquatic insects is sometimes questioned but there seems to be an almost universal agreement that many of the higher orders, including the Lepidoptera, are preëminently terrestrial in organization and that their aquatic representatives display an evolution superimposed upon a terrestrial background. These insects, invading water, had certain vital problems to solve, respiration being among the first, and the diverse but successful adaptations offer interesting material for study.

The writer has found the aquatic Lepidoptera favorable for the study of larval adaptations for the following reasons:

1. While the Lepidoptera constitute a large, well-defined order, the insignificant number of aquatic forms made it possible to examine most of the American species.

2. Although inconsequential numerically, they manifest adaptations to aquatic life as perfect and as diverse as many of the more conspicuous groups.

3. Heterogeneity in the methods of solving aquatic problems may appear even among species of the same genus.

4. The abundance of some species in certain environments has provided ample material for extensive observations and experiments.

5. This group of aquatic insects is practically unstudied from the morphological, physiological and ecological aspects.

Excluding the semi-aquatic forms, aquatic Lepidoptera fall into two general classes when considered from the point of view of larval respiration: (1) Those which are devoid of any special respiratory organs, and secure the requisite oxygen from the atmosphere by some physiological adaptation, from the dissolved supply in the water, or through a combination of both; and (2) those with special morphological devices in the form of gills. In the first class belong such forms as *Bellura melanopyga*, *Nymph-*

¹ Contribution from the University of Michigan Biological Station, and the Zoological Laboratory, University of Michigan.

ula icciusalis, and *Pyrausta penitalis*, while *Nymphula maculalis*, *N. obscuralis*, and *Cataclysta fulicalis* are typical representatives of the second.

ACKNOWLEDGMENTS

The writer wishes to express his indebtedness to Professor S. A. Forbes, Professor J. G. Needham and Mr. J. T. Lloyd for certain materials specified on later pages. Use has been made of data accumulated by two of the writer's graduate students, Miss Ethelynn Hopkins and Mr. Jennings Hickman. During the summer of 1919, Miss Hopkins studied briefly the tracheation of certain instars in *Nymphula maculalis* and *Nymphula icciusalis*, using living material. Later, Mr. Hickman examined the gills of *Nymphula maculalis*, using serial sections of preserved specimens. The work of both of these students has been repeated and extended by the writer.

MATERIAL

The principal objects of the work on which this paper is based were to determine the structure and mode of function of the respiratory organs developed in this group; to study the degree of change from the original terrestrial organization of the larvae; to get some measure on the efficiency of special organs; to examine the various instars with respect to the respiratory problem; to test certain doubtful statements in the literature; and if possible to determine something as to the course of evolution in such a limited group.

In order to accomplish these ends it was necessary to study as many representatives of the two above mentioned classes of aquatic Lepidoptera as possible. Owing to the abundance of several species in the vicinity of the University of Michigan Biological Station, repeated observations and experiments have been made during the past four summers. Some of the necessary preliminary work on habits and life-history has been published already (Welch, 1914, 1915, 1916, 1919). The material used most extensively in the present work is as follows:

Non-gilled Larvae

Nymphula icciusalis Wlk.—This species occurs abundantly at Douglas Lake, Michigan, and several instars have been studied in both living and preserved form.

Nymphula oblitalis Wlk.—Preserved material of the larvae of this species was secured from the collections of the Illinois Natural History Survey through the courtesy of Professor S. A. Forbes. The life-history of this species was described by Hart (1895, pp. 176–180) and the specimens used were from Hart's collections and identified by him. Living material has not been available.

Nymphula sp.—Larvae of a species of *Nymphula* occasionally appeared at Douglas Lake on the leaves of the yellow water-lily but it has not been bred to the adult stage and its specific identity is not known.

Gilled Larvae

Nymphula maculalis Clem.—Living material in all stages of the life-history including larval instars is available in abundance at Douglas Lake, Michigan, during July and August. Observations were also made on living specimens at Lake Oneida, N. Y., during the summer of 1916. Liberal use was made of preserved material in connection with the morphological work on the spiracles and gills. The biology of this species (Welch, 1916) has already been described.

Nymphula obscuralis Grt.—Preserved larvae were received from the Illinois Natural History Survey. Hart (1985, pp. 167–173) reported on the life-history of this form and the specimens sent to the writer were from Hart's collections and identified by him. No living material was available.

Cataclysta fulicalis Clem.—Preserved larvae were sent to the author by Professor J. G. Needham and Mr. J. T. Lloyd, from collections made in the vicinity of Ithaca, New York. These gilled caterpillars have been described by Lloyd (1914, 1919) and are conspicuously different from the non-gilled *Cataclysta* forms in other parts of the world. Living material was not available.

THE TRACHEAL SYSTEM

Since the gilled caterpillars represent the greatest progress in the direction of special aquatic respiratory adaptation and since the gap between the gilled and non-gilled forms is without intergrades, it was of interest to compare the tracheal systems of species representing these two classes and to seek light on the following questions: (1) Do the non-gilled forms possess a system of tracheation built on the plan of terrestrial lepidopterous larvae? (2) Do the gilled forms have the same system of tracheation found in the non-gilled aquatic forms? or (3) Has the acquisition of gills been accompanied by noteworthy changes in the fundamental tracheation?

These questions are significant in connection with certain facts of general habits and life-history. There is little or no doubt at present that the non-gilled forms once included in the genus *Hydrocampa* and the gilled forms once included in the genus *Paraponyx* really comprise one genus, *Nymphula*, and they are usually so treated. In Douglas Lake and other similar lakes studied by the writer, it is commonplace to find the gilled caterpillars and non-gilled caterpillars thriving side by side in the same vegetation beds, subject to identical environmental factors. In *N. maculalis* the first instar is devoid of gills, these appearing only in the second instar, when forty gill-filaments come into existence. The gill-filaments

increase in number with each succeeding molt until the final larval instar has an equipment of over four hundred.

Non-gilled Larvae

A study was made, using living material, of the arrangement and distribution of the tracheae in the different larval instars of *N. icciusalis*, giving special attention to the early instars and the full-grown larva. Taking into account only such tracheae as appear under the higher powers of the binocular microscope and the medium powers of the compound microscope, and using only fresh preparations from which none of the air had been lost, it was possible to diagram the fundamental structure of the tracheal system and to make comparisons not only in the different instars but also with other species.

It has thus been found that the ground plan of arrangement and distribution of tracheae is essentially that which characterizes the terrestrial larvae. Deviations and minor variations appear but none of them seem to bear any significant relation to the acquired habits of the larvae. No important changes of any kind appear as later instars are reached.

Gilled Larvae

Detailed studies of tracheation in the larval instars have also been carried on with *N. maculalis* using fresh, living material. In the first instar the system is almost a duplicate of that in the first instar of *N. icciusalis*. In the second, the appearance of gills is accompanied by no consequential change, the gills being supplied by short direct branches from the main longitudinal tracheae. Also in the rapidly increasing gill complexity of the later instars there is no deviation which the writer can recognize as having any significance in relation to the aquatic habit or to the acquisition of gills.

The results of this part of the work seem to show that fundamentally these aquatic caterpillars have retained the original terrestrial form of body tracheation and that the gill tracheation is a system superimposed upon the one already present in the whole group. Gills have therefore been developed with a minimal change of the original tracheation. It would appear, if these conclusions are well taken, that the larval type represented by that of *N. icciusalis* is the older one phylogenetically and that the gilled caterpillar has a more recent origin.

STRUCTURE OF THE GILLS

A detailed study of the structure of the gills in all of the species available was made by means of longitudinal and transverse sections of preserved material and by the examination of living material, whenever the latter could be secured. The relative transparency of the living material, especially when submerged in dilute glycerine, or some of the oils used for

clearing made it possible to study certain features more readily than in sections, particularly the distribution of tracheoles. Sections cut 6 microns thick and double stained in haemotoxylin and eosin gave satisfactory results. High magnification was often required for the examination of sections, particularly for the study of the histological features, and for the more critical and difficult features a 1.9 mm. oil immersion fluorite objective was used in connection with a monobinocular microscope.

The Gill-wall

A study of the gill-wall shows that it is essentially a continuation of the body-wall, having the same set of layers. In order to determine whether any differences in thickness appear, a number of measurements were made of the various layers of the gill and of adjacent portions of the body-wall, the averages being given in the following table. All of these measurements were made on specimens in the last larval instar. Measurements in this and succeeding tables are expressed in fractions of a millimeter. The terms *epidermis* and *dermis* are used instead of the *primary cuticula* and *secondary cuticula* of some authors.

	Epidermis	Dermis	Hypodermis	Total
<i>N. maculalis</i>				
Gill-wall.....	0.0004	0.0056	0.0017	0.0077
Body-wall.....	0.0004	0.0115	0.0027	0.0146
<i>N. obscuralis</i>				
Gill-wall.....	0.0012	0.0017	0.0009	0.0038
Body-wall.....	0.0005	0.0037	0.0010	0.0052
<i>C. fulcalis</i>				
Gill-wall.....	0.0037	0.0075	0.0025	0.0137
Body-wall.....	0.0037	0.0190	0.0033	0.0260

It thus appears that there is a distinct reduction of thickness in the walls of the gills as compared with the body-wall, this reduction occurring mainly in the dermis. The basement membrane is so delicate that it has been left out of account in all measurements of body-wall and gill-walls.

The Gill-cavity

The interior of each gill is merely a cavity (Pl. X, fig. 6) enclosed by the walls described above and containing certain structures to be discussed in another connection. This cavity extends continuously from base to tip and lacks completely, in the species examined, the alveolar type of tissue which appears within the gills of some insects. The size of this

cavity depends entirely upon the dimensions of the gill as a whole and has a direct connection with the haemocoel, in fact, it is a continuation of the haemocoel. In the *Nymphula* group, the gills are relatively large, have a spacious gill-cavity, and the passage from the haemocoel to the gill-cavity is broad, while in *Cataclysta fulicalis* the gill as a whole is small and slender, the wall thick, the gill-cavity much reduced, and the passage from the haemocoel to the gill-cavity often smaller in diameter than that of the gill-cavity. For example, in one series of eighteen measurements the average diameter of the gill-cavity was 0.0054 mm. while the average diameter of the opening into the haemocoel was 0.0039 mm.

Contents of the Gill-cavity

Tracheae

Nymphula maculalis.—As previously mentioned, the gills in this species are supplied with tracheal branches arising directly from the adjacent main longitudinal tracheae. Since the gills become branched in later instars, the supplying tracheae branch correspondingly. Each filament, therefore, has one main trachea, axial in position, which extends from the base almost to the tip, gradually decreasing in diameter distad. A very few instances of two tracheal branches entering a gill-filament were observed, both extending well towards the tip of the filament and both giving rise to tracheoles.

The origin and distribution of the tracheoles were best studied in living material, although certain data were confirmed by means of serial sections. At frequent but irregular intervals (Pl. XI, figs. 9, 10, 11) along the supplying trachea short tracheoles arise singly, extending ectad to the inner surface of the gill-wall and giving off numerous fine branches, all of which break up into very minute tracheoles and have a rather definite arrangement as follows. These tiny tubes all extend longitudinally, proximad and distad, very near or in contact with the ental surface of the gill-wall, and approximately parallel to each other, so that the periphery of the gill-cavity is bounded by a thin zone composed of countless, minute, parallel tracheoles. The terminal tracheoles of each individual tuft intermingle with those of the adjacent tufts but also in an approximately parallel fashion. All attempts to determine the character of the terminations of these tracheole endings, using the best preparations and the highest magnifications, have thus far been futile. In living material and in whole mounts they appear to unite with the basement membrane and serial sections confirm this conclusion, but nothing further can be said as to the exact relation to the gill-wall. No tracheoles were found lying free in the gill-cavity. The profusion of these tracheoles, intimately related to the entire inner surface of the gill-wall, points definitely to the principal function of these body projections.

Nymphula obscuralis.—Since it has not been possible to study living material of this species, the tracheation of the gills cannot be so definitely described. However, serial sections show a type of structure closely resembling that of *N. maculalis*. It is probable that both species have systems which are very similar.

Cataclysta fulicalis.—Serial sections including all parts of the body of the larva show no tracheation (Pl. X, fig. 5) of the gills. No branches of the body tracheal system approach the bases of these organs and in no sense are they to be regarded as *tracheal* gills.

Body Fluids

In living specimens of *N. maculalis*, it is easy to observe the movement of fluid, not only within but also into and out of the gill-cavity, thus giving added proof of the continuity of this cavity with the haemocoel. Blood corpuscles can be detected in this fluid. Sections confirm the observations on living specimens, showing that the gill-cavities invariably contain haemocoel fluids. Preserved material of *N. obscuralis* yielded similar results.

The small attenuated gill-cavities of *Cataclysta fulicalis* contain only the remains of fluid originating from the haemocoel.

DISCUSSION

It thus appears, from the point of view of structure alone, that two distinct gill types occur in aquatic larvae of Lepidoptera: (1) combination tracheal-blood gills, and (2) blood gills.

As already pointed out, the profusion of tracheoles in each gill-filament in *N. maculalis* indicates the respiratory nature of these organs. With its equipment of over four hundred gill-filaments the mature larva apparently has more than ample provision for respiration, especially since these larvae live in surface water rich in dissolved oxygen, and often in vegetation beds which contribute additional oxygen. This gill equipment is also striking in view of the fact that certain non-gilled *Nymphula* larvae thrive in identically the same external conditions. *Nymphula obscuralis*, according to Hart (1895, p. 170), has an average of four hundred and eighty-four gill-filaments. In *Cataclysta fulicalis*, the number is smaller, the full-grown larva having about one hundred and twenty unbranched gills.

Both blood gills and tracheal-blood gills are known to occur in limited numbers in other orders of insects. There is no special difficulty in understanding the mode of functioning of the ordinary tracheal gill, but in the combination described above and in the blood gill of the *Cataclysta* type, certain problems arise, first of which is the nature of the relation, if any, of the blood (the body fluid which circulates in the gill-cavities) to the transportation of oxygen. In the blood gills of certain chironomids, the

blood contains haemoglobin and with this carrier present the gills have definite significance. In the larvae of *N. maculalis* and in certain other insects having similar gills, some carrier other than haemoglobin seems necessary to enable these structures to function as gills. The actual discovery of invisible carriers has not yet occurred. Muttkowski (1920, 1921a, 1921b, 1921c) suggests that possibly haemocyanin may constitute such a carrier. Rose and Bodansky (1920) demonstrated the presence of copper in a number of marine organisms and Muttkowski (1921a) found it in a large number of animals representing six phyla. The last named investigator holds that "Copper is found in insect blood in quantities comparable to that of crayfish blood. Its rôle is therefore interpreted as being identical,—namely that it serves as the nucleus of a respiratory protein,—hemocyanin. Insects, therefore, have two sources of oxygen,—atmospheric air led directly to the tissues by way of the tracheae, and fixed oxygen carried by the respiratory protein of the blood." Possibly this is a hint in the right direction and invisible oxygen carriers in insect blood may soon be identified.

Since the circumstances seem to demand the presence of some oxygen carrier, the question arises concerning the mode of functioning of the combination gill. Does such a gill have two separate and distinct methods of securing and distributing oxygen? The position and distribution of the tracheoles are such that there seems to be no ground for assuming any relation to the blood as an intermediary between them and incoming oxygen. Perhaps the tracheal system might function as completely if the gill-cavity were filled with alveolar tissue instead of blood. On structural grounds alone, it appears possible that two distinct methods could exist side by side. It might be suggested that in *N. maculalis* and *N. obscuralis* the gills are really tracheal gills and that the presence of blood in the gill cavity is entirely incidental, but such a suggestion loses weight when *C. fulicalis* is considered since its gills, if they function at all, must do so through the intermediation of the blood. It has not been proven absolutely that these lateral outgrowths in *C. fulicalis* are functional gills and as respiratory organs they might be questioned completely. Such a view would render similar organs in other orders of insects devoid of respiratory significance and, pending further investigation, it would seem that circumstantial evidence points rather definitely to the conclusion that these organs do function in respiration.

Judging entirely from the structure of these gills, a contrast appears between the *Nymphula* group and *Cataclysta* which may indicate difference in degree of efficiency. In the former, the larger number of gills, the rather spacious gill-cavities, the thin gill-walls, and the profuse tracheation all suggest an efficient equipment. In the latter, however, with only about one hundred and twenty gills, with the very small gill-cavities connected

with the haemocoel by still smaller lumina, with no traces of tracheation, and with the conspicuously thick gill-walls, the effectiveness of the system seems much smaller.

The presence and absence of gills within the genera *Nymphula* and *Cataclysta* and the existence of distinctly different gill types in these two closely related genera give added support to the theory of the independent origin of the various aquatic insects, emphasizing the fact that in these animals types of adaptation and genetic relationship may have no close correlation.

THE SPIRACLES AND CONNECTING TRACHEAE

The secondarily acquired nature of aquatic habits and structures naturally directs attention to the character of the spiracles. In the gilled forms, is the gill system superimposed upon an unmodified holopneustic tracheation, or have modifications occurred leading towards suppression of the spiracular equipment? In the non-gilled forms which lead a submerged existence, has the characteristically terrestrial holopneustic tracheation been modified? A common statement appears in the literature to the effect that many nymphs and larvae living in water have apneustic tracheation, breathing directly through the skin or by means of gills. It is also pointed out that between the completely apneustic and the typical holopneustic tracheation a variety of intermediate stages exists. The gilled larvae of certain *Nymphula* species have been described as having apneustic tracheation in which the spiracles are closed, and the spiracular branches (stigmatal branches) have become solid cords. The writer has searched in vain for any thoroughgoing morphological work bearing on this subject. Among workers who have studied Old World species of *Nymphula* three of the most recent might be mentioned. Rebel (1899) made some studies on *Nymphula* (*Paraponyx*) *stratitota* and states that the tracheation is apneustic. Portier (1911) in a voluminous paper dealing with several aquatic insects, carried on some physiological experiments with larvae of *Nymphula stratitota* and claims to have shown that (a) larvae submerged for five minutes in olive oil colored with alcanine showed no oil in the tracheae; (b) that under the binocular microscope the spiracular trunks did not have the aspect characteristic of air filled tracheae but looked like heavy cords; (c) that larvae suffered no effects from submergence in oil, but a small geometrid larva so treated became inert and oil was found in its tracheae; (d) that larvae were perfectly normal after fifteen minutes submergence in soapy water, but the geometrid larva so treated became apparently dead in three minutes; (e) that larvae immersed in olive oil, ether, and alcanine became anesthetized after one minute, but microscopic examination showed no penetration of the colored oil, recovery occurring when returned to water; (f) that larva treated as in (e) for

twenty hours did not show the tracheal system invaded; (g) that a larva placed under reduced air pressure showed bubbles of gas gradually form on the surface of the body where the integument is thinnest but none formed about any of the spiracles. From these experiments and without morphological confirmation Portier concluded that the spiracles of the larva of *N. stratiotata* are closed and functionless. Wesenberg-Lund (1913, p. 126) states that in *N. ("Paraponyx") stratiotata* the spiracles are functionless but no evidence is given in support of this conclusion.

OBSERVATIONS AND EXPERIMENTS OF NYMPHULA MACULALIS

Attention was first directed to the spiracles by evidence that these larvae can exist out of water for considerable periods of time. While it is common for the pupa to be formed on the lower, submerged surface of a water-lily leaf, the full-grown larva sometimes crawls out of the water, onto the upper leaf-surface and there forms the pupa. In order to construct the silken covering, tie down the case to the leaf, and transform into a pupa, a considerable period of time must be spent out of water. Mature larvae placed in containers without food and with little or no moisture often lived from four to eight days. Larvae, in containers with food material and just enough water to keep the surrounding air moist, were allowed to gradually dry up. In such cases the gills became dried and black, but the larvae lived about fourteen days. It is thus evident that these caterpillars can exist for days apart from water and respire by means other than gills. Furthermore, it seems unlikely that this can be accounted for on the basis of cutaneous respiration. These results led to a critical morphological examination of the spiracles and their connecting tracheae in *N. maculalis*, a study which was later extended to include all of the strictly aquatic caterpillars available for examination.

MORPHOLOGY OF THE SPIRACLES AND CONNECTING TRACHEAE

A detailed morphological study of the spiracles and their connecting tracheae was made on serial sections, cut six to seven microns thick, and double stained. Both transverse and longitudinal sections were used and all critical points determined with a modern monobinocular microscope equipped with a 1.9 mm. fluorite oil immersion objective. Since the spiracular aperture may be narrower in one dimension than the other, the long dimension being transverse to the long axis of the body, measurements on transverse sections might lead to error if not checked on longitudinal sections. In all such cases the measurements were made from the edges of the opening, not including accessory structures, and in such a way as to give an average of the two principal diameters of the aperture. On the other hand, the connecting tracheae are practically cylindrical, thus making it possible to record measurements from any section passing

through the center of the lumina. Mature larvae were usually used, although sections of earlier instars were examined from time to time.

In this connection it should be pointed out that, as usual in lepidopterous larvae, the spiracles on the meso- and metathorax are absent in all of the species examined. In the following tables percentage of decrease, wherever expressed, is calculated by using as the standard of comparison the dimensions of the largest spiracle and connecting trachea (usually those of the second abdominal segment) of the series. It must also be understood that this is merely a convenient way of comparing the degree of reduction of the other spiracles and tracheae and is not intended to imply that even the largest may not have undergone some reduction themselves. Since there is no way of determining the diminution of the largest spiracle, if it has been reduced, it would not be possible to express the *true amount of reduction* of the other spiracles on the same individual.

Nymphula maculalis

Examined externally, under magnification, nine pairs of spiracles are observable on segments 1, and 4-11. All are minute and inconspicuous except those on 5, 6, and 7 which are distinctly larger. The following table comprises one set of diameter measurements which is representative of all others made in this work:

Segments	Spiracles	Lumen of Spiracular Tracheae	Percentage of Decrease	
			Spiracles	Tracheae
1	0.0050	0.0011	85.5	96.0
2
3
4	0.0051	0.0011	85.2	96.0
5	0.0345	0.0276	0.	0.
6	0.0322	0.0253	0.66	0.8
7	0.0322	0.0184	0.66	33.3
8	0.0119	0.0011	65.5	96.0
9	0.0085	0.0017	75.3	93.8
10	0.0068	0.0017	80.2	93.8
11	0.0051	0.0013	85.2	95.2

THE LARGER SPIRACLES AND CONNECTING TRACHEAE

In the larger spiracles the outer margin of the aperture bears a complete set of elongated, closely set, chitinous spines (Pl. X, fig. 1) which have a rich yellow color when viewed under magnification. These spines form an almost continuous marginal guarding device, the free ends converging so that the form of the whole is that of a truncated cone. The free ends of the spines mark the periphery of an aperture which is much

smaller than the spiracle itself. The external cuticula extends into the lumen of the spiracular trachea, lining it for the entire length. However, certain modifications appear chief among which are the distinct reduction in thickness and the numerous filiform chitinous projections which extend into the lumen. A continuation of the hypodermis of the body-wall constitutes the major part of the wall of the spiracular trachea and shows no significant changes in structure.

The short connecting trunk is terminated at its ental end by a well-developed closing apparatus (Pl. X, fig. 4), composed essentially of a closing bow, a closing band, a closing lever, and an occlusor muscle. The closing bow is a chitinous, crescentic band (Pl. XI, fig. 7) lying in the lining of the lumen and extending through one-half of the total circumference. From one end of the closing bow a similar band continues around to a point about opposite the middle of the closing bow where it meets the end of the closing lever. The closing lever is located at right angles to the lumen and projects radially for its entire length, covered by an extension of the hypodermal wall. A short, broad occlusor muscle extends from the end of the closing lever diagonally to the free end of the closing bow. It thus appears that the chitinous band formed in this way is absent for about one fourth of the circumference of the lumen, thus failing to form a complete ring. Thus far it has been impossible to determine the exact relation of the chitinous parts of the closing apparatus to each other. Studies were made using thin serial sections in all the principal planes; also by dissecting out a portion of the body of the larva containing a large spiracle and its related tracheal parts, placing them on a slide in strong potassium hydroxide solution and boiling by holding the slide over a small flame. By the use of this last named method the soft parts were all removed leaving only the chitinous portions. Difficulties in tracing out certain minute portions of this closing apparatus have not been entirely overcome either by the kind of preparation or by high magnification. It appears, however, that the chitinous band, forming the closing lever and the closing bow, is one continuous structure.

From the outermost end of the closing lever a long muscle band extends diagonally to the body-wall. On the opposite side a similar muscle extends from a point near the origin of the occlusor muscle diagonally to the body-wall. Beyond the closing apparatus the lumen opens directly into that of the main longitudinal tracheal trunk.

The Smaller Spiracles and Connecting Tracheae.—The spiracles on segments 1, 4, 8, 9, 10, and 11 show a marked reduction in size, in fact, they are so small that magnification is necessary to locate them definitely. Structurally, these spiracles and their connecting tracheae differ markedly from those on 5, 6, and 7. At the margin of each spiracle there appears, instead of a thick set crown of chitinous spines, a solid, continuous, chiti-

nous rim (Pl. X, fig. 3) which projects distad from the body-surface. It would appear that in the process of reduction the spiny crown of the original large spiracles became fused into one continuous margin. At the base of the tiny cup thus formed the lumen becomes reduced to an extremely fine canal which extends without change in diameter to the closing apparatus located well within the haemocoel. This canal is lined throughout by a uniform, thin extension of the external cuticula, but the hair-like projections characteristic of the large spiracular trunks are here entirely wanting. As will appear in the table, the lumen is very minute, but by means of thin, serial sections and high magnification it has been possible to demonstrate that it is *open* throughout its course. The bulk of the wall of the spiracular trachea is composed of an extension of the hypodermis of the body-wall, but it also has become reduced, being about one-half the thickness of the same layer in the larger spiracular tracheae. The connecting trachea has not changed in length and terminates in a closing apparatus similar to that described for the larger spiracular tracheae, except that it also has become considerably reduced in size. All of the parts are represented, however, and the whole closing contrivance has every appearance of being completely functional.

It also appears that reduction in the small spiracles is not uniform, but gradually increases posteriorad. This, however, does not seem to hold for the connecting tracheae.

Nymphula obscuralis

An examination of *Nymphula obscuralis* showed a condition very similar to that in *N. maculalis*. While the following diameter measurements were taken from a single mature larva, they are representative of those for other larvae. The structural features of the spiracles, the connecting

Segment	Spiracles	Lumen of Spiracular Tracheae	Percentage of Decrease	
			In Spiracles	In Tracheae
1	0.0074	0.0005	66.6	97.3
2
3
4	0.0074	0.0005	66.6	97.3
5	0.0222	0.0185	0.0	0.0
6	0.0222	0.0185	0.0	0.0
7	0.0222	0.0185	0.0	0.0
8	0.0074	0.0024	66.6	87.0
9	0.0074	0.0017	66.6	90.8
10	0.0074	0.0017	66.6	90.8
11	0.0074	0.0005	66.6	97.3

tracheae, and the closing apparatus, are also so similar that no description is necessary here.

Cataclysta fulicalis

In *Cataclysta fulicalis* the spiracles and the connecting tracheae differ from those of the gilled *Nymphula* caterpillars in that no reduction of any kind appears, all being of practically uniform size and structure. Some variation occurs but it is slight and apparently insignificant. The circle of guarding spines at the outer periphery of the spiracle is less convergent (Pl. X, fig. 2) than in the gilled *Nymphula* larvae, thus forming a wider aperture. Structurally, the spiracles, connecting tracheae, and the closing apparatus are similar to those of the gilled *Nymphula* group, slight but inconsequential deviations being present. The following table includes a typical set of diameter measurements made on one specimen:

Segment.	1	2	3	4	5	6	7	8	9	10	11
Spiracle..	0.026	0.029	0.028	0.028	0.03	0.035	0.031	0.031	0.031
Lumen of trachea.	0.03	0.033	0.043	0.035	0.035	0.033	0.040	0.038	0.038

When all measurements were averaged, the spiracles and their connecting trunks were found virtually uniform in size. The larvae of *Cataclysta fulicalis* have thus acquired a system of gills without accompanying changes in the spiracular system, the latter being as completely open morphologically as any terrestrial caterpillar.

Nymphula oblitalis

The spiracles and connecting tracheae are distinctly open and show no evidence of definite reduction. A certain variation appears, as will be noted in the following table, but even the smallest found is far above the corresponding reduced structures in *N. maculalis* and *N. obscuralis*. In the following representative diameter measurements taken from the record of one mature specimen, nothing is especially noteworthy except the large spiracle and connecting trachea on the fourth segment. Whether the difference in size is an evidence of a slight reduction of spiracles and connecting tracheae in the posterior segments is uncertain. The principal features of the closing apparatus are shown in figure 8.

Segment.	1	2	3	4	5	6	7	8	9	10	11
Spiracle..	0.032	0.044	0.04	0.04	0.04	0.032	0.032	0.036	0.04
Lumen of trachea.	0.028	0.064	0.028	0.028	0.024	0.020	0.020	0.020	0.036

Nymphula sp.

A non-gilled form, distinctly different from any other used in this work but whose specific identity is unknown, was examined in this connection. Sections showed all of the spiracles to be distinct, well-developed, open, and approximately uniform in structure and size, the same being true of the connecting tracheae. Average measurements for the whole series are as follows, the extreme variations deviating very little from the average: Diameter of the spiracles, 0.05569; diameter of the lumina of the tracheae, 0.0527.

Experiments

In order to demonstrate experimentally the open condition of the spiracles and connecting tracheae and check the morphological results, certain experiments were made on the caterpillars *N. maculalis*. Larvae dropped into hot water invariably give off one or more bubbles of gas from the large spiracles on segments 5-7, thus showing definitely the open condition of these spiracles and their connecting tubes. No gas was given off from the reduced spiracles, but it does not follow that such failure is due to complete closure since the amount of gas in the tiny lumina is extremely slight and the expansion of the heated gas in the longitudinal tracheal trunks would be more likely to be released at the larger and more open spiracles on segments 5-7.

It was found that the larvae of *N. maculalis* could live in commercial kerosene for 6-7 hours. They were then submerged in kerosene colored with Sudan III. Since the translucency of the body-wall made it possible to trace much of tracheal system, it was easy to examine the spiracular connections at any time and to follow the entrance of the colored liquid into the larger spiracles. Positive evidence that these spiracles are morphologically open was thus repeatedly secured. This penetration, into the large spiracles, occurred within an hour, but entrance into the smaller ones was much slower although ultimately the colored liquid was observed in some of the connecting tracheae.

DISCUSSION

It thus appears, at least in *Nymphula maculalis*, *N. obscuralis*, and *Cataglyphis fulicalis*, that in spite of the gill development, the tracheal system of the larvae is morphologically open. The reduction of certain spiracles and their connecting tracheae in the first two is definite and striking but has not progressed to the place where the lumina and apertures are completely closed. Since the previous work on this subject has been done on unavailable foreign species it is not possible, on the basis of the present work, to absolutely refute the statements made in the literature, but the writer is inclined to suspect strongly that what has been found

in *N. maculalis* and *N. obscuralis* is likewise true of *N. stratiotata* and other foreign gilled representatives of that genus. Portier's results (1911), secured as they were without any attempt at critical morphological work, cannot be regarded as conclusive. However, it is not inconceivable or impossible that certain species might have progressed to the point of closing the spiracular system but if such a condition does exist it should be demonstrated more convincingly than has heretofore been done.

In regard to the functioning of these reduced spiracles and tracheae, no serious question can be raised concerning the larger ones on segments 5-7 in *N. maculalis* and *N. obscuralis* since their size, open character of the connecting tracheae, and structure of the closing apparatus all indicate the possibility of normal activity. In spite of the small diameter of the more reduced spiracles and tracheae, the writer has thus far found nothing which would prohibit at least a limited functioning of these organs. That air will pass through pores and tubes of smaller diameters than those of the organs under discussion is now known, and many of the very minute insects known to have a typical holopneustic type of tracheation have openings and tracheae no larger than the reduced ones of the gilled *Nymphula* caterpillars. Likewise if the minute tracheoles of the tracheal system which are less than one micron in diameter can transport atmospheric gases, failure of the reduced spiracles and connecting tracheae to function would have to be due to some feature other than the structure of the tubes themselves. That there would no difficulty in the ventilation of such a system has recently been shown by Krogh (1920a; 1920b) since in small forms diffusion alone will provide the necessary oxygen transportation, although it may be assisted by respiratory movements of the animal, if the latter are manifested.

In certain insects having apneustic tracheation the spiracles and connecting tracheae are said to be temporarily open at the time of molting. This, however, does not account for the open character of the gilled larvae of *Nymphula* since sections of specimens in various parts of the stadia involved were studied and all yielded the same result.

There seem to be no reasons for assuming that open spiracles and open connecting tracheae are necessarily inimical to larvae existing in water since certain well known forms, as for example, *Bellura melanopyga*, have complete sets of open spiracles, yet are related to the water in such a way that most or all of these organs are submerged for long periods of time. It is not unlikely that still other aquatic larvae, thought to have true apneustic tracheation, will be found to possess morphologically open spiracles.

What part these open spiracles play in the life of the forms involved is difficult, at present, to specify. As has been pointed out, the gilled *Nymphula* larvae can pass extended periods of time out of water, at least

during the last larval stadium. This indicates functioning of the spiracles, since it is unlikely that the requisite amount of oxygen could be secured by cutaneous means alone, especially after the surface of the body became dry. In regard to submergence, it is possible that a provision against penetration of water into the tracheal system is afforded in the combination of structures present. The marginal crown of guarding spines or their derivatives, if hydrofuge in character, may constitute an efficient protection. It is also possible that the well developed closing apparatus plays some part in this connection.

GENERAL CONSIDERATIONS

From the point of view of the respiratory mechanisms involved, the true aquatic Lepidoptera comprise a heterogeneous assemblage, including those, on the one hand, which have made no morphological advance towards the aquatic life, and those on the other hand, which manifest highly developed morphological adaptations of an aquatic sort. These adaptations involve, in the most complex type, the addition of structurally complex gills, and the marked reduction in size of spiracles and connecting tracheae. It should be noted that apparently no advantage has accrued to the possessors of the complex adaptation since in all of the situations examined by the writer the non-gilled larvae, having the unmodified tracheal system of a terrestrial type, have had every appearance of being as successful in the aquatic medium as their more specialized relatives, often existing in identically the same environment and offering an interesting parallel in the solution of the same problems by very different means. There would seem to be a considerable advantage in the possession of over 400 gill-filaments and a set of reduced spiracles, particularly in those cases where the size of the body proper is virtually that of the associated non-gilled forms. As previously mentioned, the structure of the gills in *Nymphula* is such that it seems almost inconceivable that they do not function as true respiratory organs. In a recent paper, Fox (1921) claims to have demonstrated that in a certain chironomid larva, oxygen is not taken up by the ventral blood gills; that the anal gills take up less than the corresponding area of the body-surface; and that most of the oxygen is received through the body-wall in general. These surprising results require confirmation and at present need not be regarded as serious ground for questioning the function of the gills in other insects.

On the basis of structure alone it might appear that the small blood gills of *Cataclysta* represent a more primitive stage in the development of aquatic respiratory adaptation than that represented in the gilled larvae of *Nymphula*. However, there is no indication that any of the *Nymphula* species have ever had blood gills only. The evolution of these larval

adaptations has apparently been a sporadic phenomenon with the extremes sometimes occurring within the confines of a single genus.

SUMMARY

1. Fundamentally, aquatic larvae of the genus *Nymphula* have retained the original terrestrial type of body tracheation in practically unmodified form. The tracheation of the gills has been superimposed upon the terrestrial type with minimal change to the latter, and the non-gilled larval type is doubtless the older one phylogenetically.

2. Gills in the aquatic Lepidoptera are all hollow outgrowths of the body-wall, the cavity being in direct communication with the haemocoel. All of the layers of the body-wall are represented but in reduced thickness, maximum diminution appearing in the dermis.

3. In all of the gilled larvae of *Nymphula* examined, the gill-cavity contains both an elaborate set of tracheae and tracheoles and a considerable quantity of body fluid, thus constituting a combination tracheal-blood gill. In the larvae of *Cataclysta fulcalis* the gills have no traces of tracheae and are thus blood gills only.

4. In the non-gilled larvae of *Nymphula* and the gilled larvae of *Cataclysta fulcalis* the tracheation is typically holopneustic, no reduction of any significance appearing either in the spiracles or their connecting tracheae.

5. In gilled larvae of *Nymphula*, a distinct reduction appears in the spiracles and their connecting tracheae on segments 1, 4, 8, 9, 10, and 11, those on segments 5, 6, and 7 being much larger and having undergone less reduction.

6. Morphological and experimental studies on gilled *Nymphula* larvae have shown that in spite of the striking reduction of some of the spiracles and connecting tracheae, the tracheation is still holopneustic, all spiracles and tracheae being morphologically open with nothing to indicate that they are functionless. While gilled representatives of foreign species of this genus have not been available, it is very probable that the statements in the literature to the effect that they have a closed tracheal and spiracular system are in error, due to insufficient study.

7. The gilled larvae of *Nymphula maculalis* may live for extended periods of time outside of water, even after the outer surface becomes dry and the gill-filaments shriveled, indicating that respiration through the spiracles is being accomplished.

8. Reduction of spiracles and possession of gills do not seem to be necessarily correlated or coexistent since in *Cataclysta fulcalis* both gills and an unreduced tracheal system are present.

9. In spite of the contrast between the gilled and the non-gilled species, the former seem to have no advantage over the latter, at least in those cases where both forms exist side by side in the same habitat.

LITERATURE CITED

- FOX, H. M.
 1921 Methods of Studying the Respiratory Exchange in Small Aquatic Organisms, with Particular Reference to the Use of Flagellates as an Indicator for Oxygen Consumption. *Journ. Gen. Phys.*, 3:565-573. 5 fig.
- HART, C. A.
 1895 On the Entomology of the Illinios River and Adjacent Waters. *Bull. Ill. State Lab. Nat. Hist.*, 4:149-273. 15 pl.
- KROGH, A.
 1920a Studien über Tracheenrespiration. II. Über Gasdiffusion in den Tracheen. *Pflüger's Archiv ges. Phys. d. Menschen u. d. Tiere*, 179:95-112. 5 fig.
 1920b Studien über Tracheenrespiration. III. Die Kombination von mechanischer Ventilation mit Gasdiffusion nach Versuchen an *Dytiscus*larven. *Pflüger's Archiv. ges. Phys. d. Menschen u. d. Tiere*, 179:113-120. 2 fig.
- LLOYD, J. T.
 1914 Lepidopterous Larvae from Rapid Streams. *Journ. N. Y. Ent. Soc.*, 22:145-152. 2 pl.
 1919 An Aquatic Dipterous Parasite, *Ginglymyia acirostris* Towns., and Additional Notes on its Lepidopterous Host, *Elophila fulcalis*. *Journ. N. Y. Ent. Soc.*, 27:263-265. 1 pl.
- MUTTKOWSKI, R. A.
 1920. The Respiration of Aquatic Insects. A Collective Review. *Bull. Brook. Ent. Soc.*, 15:89-96, 131-141.
 1921a Copper in Animals and Plants. *Science (N.S.)*, 53:453-454.
 1921b Studies on the Respiration of Insects. I. The Gases and Respiratory Proteins of Insect Blood. *Ann. Ent. Soc. Am.*, 14:150-156.
 1921c Copper: Its Occurrence and Rôle in Insects and Other Animals. *Trans. Am. Micr. Soc.*, 40:144-157.
- PORTIER, P.
 1911 Recherches Physiologiques sur les Insectes Aquatiques. *Arch. d. Zool. Exp.*, (5), 8:89-379. 4 pl. 67 fig.
- REBEL, H.
 1899 Zur Kenntnis der Respirationsorgane Wasserbewohnender Lepidopteren Larven. *Zool. Jahrb.*, abt. f. Syst., 18:1-26. 1 pl.
- ROSE, W. C. and BODANSKY, M.
 1920 Biochemical Studies on Marine Organisms. I. The Occurrence of Copper. *Journ. Biol. Chem.*, 44:99-112.
- WELCH, P. S.
 1914 Habits of the Larva of *Bellura melanopyga* Grote (Lepidoptera) *Biol. Bull.*, 27:97-114. 1 pl.
 1915 The Lepidoptera of the Douglas Lake Region, Northern Michigan. *Ent. News*, 26:115-119.
 1916 Contribution to the Biology of Certain Aquatic Lepidoptera. *Ann. Ent. Soc. Am.*, 9:159-187. 3 pl.
 1919. The Aquatic Adaptations of *Pyrausta penitalis* Grt. (Lepidoptera.) *Ann. Ent. Soc. Am.*, 12:213-226.
- WESENBERG-LUND, C.
 1913 Wohnungen und Gehäusebau der Süsswasserinsekten. *Fortschr. d. Naturwissensch. Forsch.*, 9:55-132. 59 fig.

Abbreviations Used in Plates

<i>bl.</i>	blood
<i>cl.</i>	closing apparatus
<i>c.t.</i>	connecting trachea
<i>d.</i>	dermis
<i>ep.</i>	epidermis
<i>gl.c.</i>	gill cavity
<i>hyp.</i>	hypodermis
<i>l.t.</i>	longitudinal trachea
<i>mu.</i>	muscle
<i>sp.</i>	spiracle
<i>t.</i>	trachea

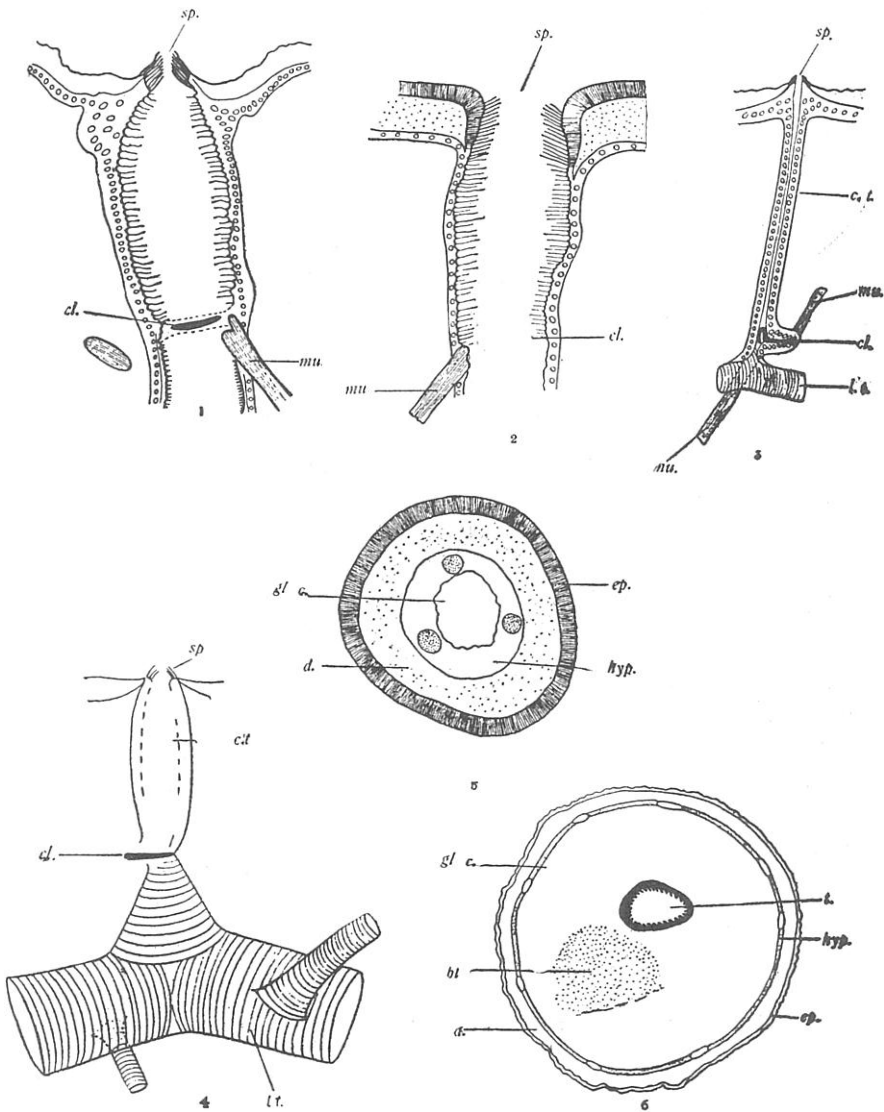


PLATE X

Fig. 1. Longitudinal section through large spiracle and connecting trachea in larva of *Nymphula maculalis*.

Fig. 2. Longitudinal section through spiracle and connecting trachea in larva of *Cataclysta fulcalis*.

Fig. 3. Longitudinal section through reduced spiracle and connecting trachea in larva of *Nymphula maculalis*.

Fig. 4. Drawing of caustic potash preparation of large spiracle and connecting trachea in larva of *Nymphula maculalis*.

Fig. 5. Transverse section of gill-filament in larva of *Cataclysta fulcalis*.

Fig. 6. Transverse section of gill-filament in larva of *Nymphula maculalis*.

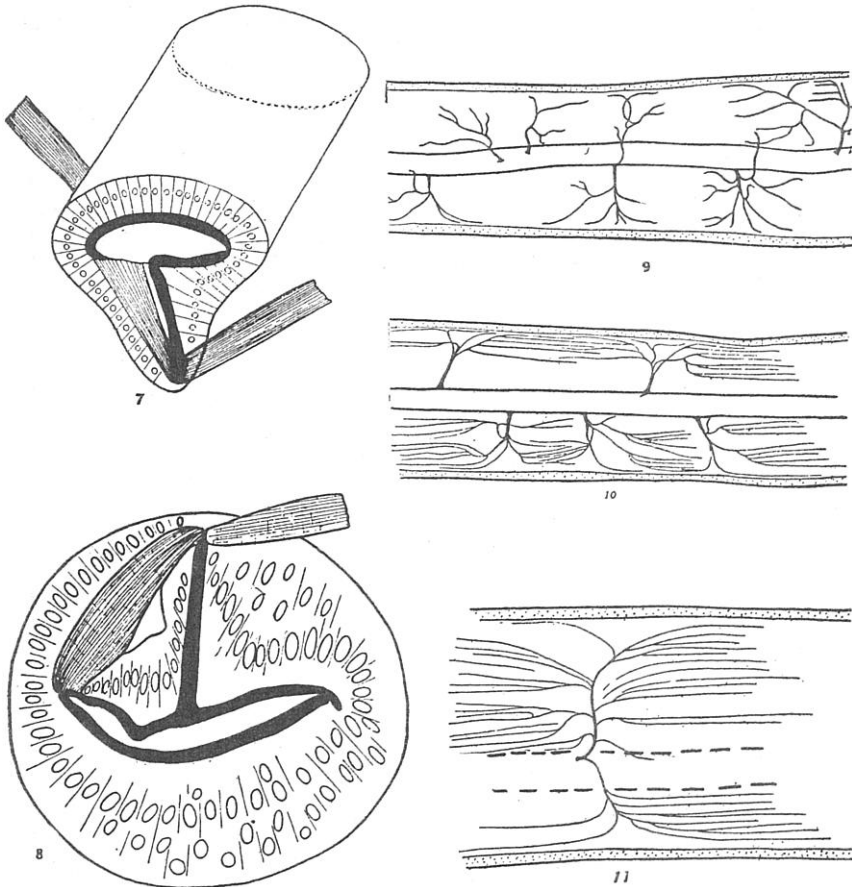


PLATE XI

Fig. 7. Transverse section at ental end of connecting trachea for large spiracle in larva of *Nymphula maculalis*, showing structure of closing apparatus.

Fig. 8. Transverse section at ental end of connecting trachea for spiracle in larva of *Nymphula oblitalis*, showing structure of closing apparatus.

Fig. 9. Camera lucida drawing from living larva of *N. maculalis* showing tracheation of gill-filament as it appears under low magnification.

Fig. 10-11. Camera lucida drawings from living larva of *N. maculalis* showing distribution and arrangement of finer tracheoles of gill-filament as they appear under high magnification.